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ENDAMOEBA BUCCALIS

I. ITS MULTIPLICATION AND PERIODICITY

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Work on these much-discussed parasites was begun with a two-fold object: (1) to find the cause of their periodic disappearance from the human mouth, this phenomenon pointing to a solution of the life-history which has not been solved; (2) to determine by tonsil smears and sections whether this endamoeba is ever intracellular.

MATERIAL

The specimens used in this study were collected entirely from one host and from one point of infection, an upper premolar tooth which was the single focus of infection for a long while. Daily record was kept of the occurrence of these parasites for a period of over five months, from May 1 to July 29, 1915, and from October 10 to December 12, 1916, as well as of health conditions of the host.

Moreover, a study of the parasite was made at various times of day and night with a view to determining whether behavior varies with these conditions.

My attention was first called to the suspected periodic disappearance of *Endamoeba buccalis* by one of my students, who was working on this parasite in the zoological laboratory of the University of Kansas during the winter of 1914. While the supply of material was usually generous, at times she reported it too scarce for study. Since then other protozoology students have reported finding it at times and being unable to do so at others. This did not seem to be wholly due to mouth cleaning, and suggested to me the possibility of migrations of the parasites into the gums or periosteum at the so-called "scarce times."

While I set out with this as a pure hypothesis, my observations during five months have convinced me that *E. buccalis* appears and disappears, tho with not so much regularity as I had at first thought; my journal shows nine scarce times in five months.

Since I have no definite proof for the ideas I am about to set forth in explanation of these migrations, I offer them only as strong circumstantial evidence, with the hope that the suggestion may serve as a good working clue.

METHODS OF STUDY

For diagnostic purposes the fresh smear containing the living material is best. A small amount of scrapings from the point where the tooth joins the gum was spread on a cover-slip slightly heated. This was then placed upon a slide containing a small drop of normal salt solution. If a prolonged study is desired, the slip can be ringed with vaselin before being placed on the slide. I found this method much better than smearing the slide and then covering with normal salt and cover-slip, or than mixing the scrapings freely with the salt solution. The natural environment is most nearly reproduced by the hanging drop method, and it may be due to this that I was able to observe behavior which, so far as I know, has not been reported for this amoeba.

Among *intra vitam* stains, neutral red differentiates the food vacuoles very quickly, but has the disadvantage of staining the whole parasite intensely in a short time. It brings out few points which the unstained, living specimen does not show. After a little experience, there is no danger of confusing the unstained endamoeba on a slide with the leukocytes. The endamoebae have a greenish refractive look which differentiates them even under the low power, where they appear smaller than a pin head. Even motion is not necessary.

For nuclear studies, further investigation of food bodies, structure of cytoplasm, etc., material was preserved, stained according to various methods of protozoa technic and compared carefully for results. Needless to say, the wet film method was adhered to thruout except in one process, and that at the very end of the Giemsa stain, just before mounting in balsam. Comparasion of a slide thus treated with one run into xylol and not allowed to dry, showed no ill effects, and the stain was better without the xylol treatment.

The three methods generally employed were:

(1) The short Giemsa. Bring the wet smear into half and half methyl and ether for 5 minutes. Transfer to a solution of Giemsa made by adding 1 cc. of stock solution to 15 cc. distilled H_2O for 8 to 10 minutes. Wash with distilled water until pink appears. Barely dry in air and mount in balsam.

This gives a beautiful differential stain for the amoebae, coloring the cytoplasm pale blue, their food vacuoles wine, and the nucleus vivid red. This stain brings out a halo of chromidia around the nucleus which other stains do not. Giemsa also differentiates leukocytes clearly

from even the smallest endamoeba because leukocyte cytoplasm stains pink and their nuclei deep lilac. Epithelial cells take a faint pink in the cytoplasm, and a deep pink nuclear stain.

(2) Fixation with the methyl-ether mixture compares favorably with the picro-mercuric-formalin method, which is recognized as one of the most satisfactory fixations for protozoa. This latter fluid used hot and allowed to stand on the smear until it has evaporated almost completely, then washed in 70 per cent alcohol and stained by Dobell's quick hematein method, gives a very clear image of the nucleus, if properly differentiated.

(3) A very similar effect is obtained by hot Schaudinn's fluid (80 parts Mg Cl_2 plus 20 parts absolute alcohol) followed by Heidenhain's iron-hematoxylin used unripened.

Mallory's stain recommended by Craig (1911) for sections was used on tonsil sections suspected of containing endamoebae, as well as on fresh smears of *Endamoeba buccalis* without satisfactory results.

REPRODUCTION

Most of the endamoebae found in smears contain nuclei in the resting stage; and this as pictured in most texts and as shown also in my own slides by the mercuro-picric-formalin fixation and either Dobell or Heidenhain's iron-hematoxylin stains, has a nuclear diameter scarcely one eighth the diameter of the amoeba, a well defined membrane, with its inner margin lined with chromatin granules, and a central body or nucleolus (Fig. *a*). A clear area surrounds the nucleus in fixed material, and since it has been impossible to study it in the living condition, it cannot be determined whether this area is due to shrinkage or is what shows as a pink halo with the Giemsa stain (Figs. *e* and *j*).

Material stained with Giemsa shows outside of this so-called nuclear membrane, and in about the region of the clear area mentioned above, a halo of rose-colored granules, which I interpret as chromidia. Every nucleus stained thus shows this, and I conclude that it is present even in the very earliest resting stage of all *Endamoeba buccalis,* but is not brought out by certain stains.

Craig (1916) believes *E. buccalis* has a primitive type of mitosis. My observations point toward a complex one. During the early stages of mitosis the nucleus enlarges and the karyosome disappears. Chromatin collects on the nuclear membrane, making an irregular border (Fig. *e*) and leaving a clear central vacuole. At times the chromatin of this phase is so organized that it gives the appearance of a spireme, except that the vacuole at the center is always present. A halo of chromidia surrounds the dense chromatin and no doubt contributes to

its formation, since it grows paler as the chromatin condenses. The chromatin condenses into four very distinct bodies and the nucleus remains in this condition longer than in others, judging by the large number found on a slide in the prophase of mitosis.

Whether these four bodies can be compared with chromosomes of higher animals, I do not know, because as soon as the spindle forms they mass and become indistinguishable. Yet they indicate very definite organization during at least part of mitosis. Figure *g* is the typical metaphase, Figure *h* the anaphase, and Figure *i* the telophase. In comparison with early stages of the nucleus these later spindle stages seem very small, the difference being due to the chromidia outside what is usually designated as the nuclear membrane. I doubt now whether

Endamoeba buccalis. For details see text.

a nucleus as represented in Figure *a* is the entire chromatin mass, since I have found chromidia in those of about the same phase stained with Giemsa (Fig. *b*).

The spindles are distinct and give the appearance of having a centrosome at the poles. The size of the nucleus varies considerably, the larger ones being lodged usually in the larger amoebae; but there are frequent exceptions to this (Fig. *j*).

I have occasionally found two equal-sized endamoebae lying in close contact and suggesting binary fission. A close study of these, however, has never revealed a dividing nucleus, but two well formed and widely separated ones. The cytoplasm was in every case completely separated. Very frequently two living endamoebae are seen gliding over each other and becoming so nearly fused that at times the most careful

observations cannot distinguish two separate animals. I have suspected that there may be an interchange of materials between two such individuals, but I have never been able to confirm Craig (1916), who says he saw streaming of cytoplasm from one to the other.

The incessant gliding of the amoebae over each other gives this appearance. This may be conjugation. I have never seen *buccalis* in the actual process of division.

I have never seen buds form and become separated from the parent cell and then develop, tho endamoebae giving the appearance of budding are frequent. At such times the cell resembles the pearl-stage of gregarines, but long enough observation has usually seen them withdrawn. It is usually an adverse condition, such as drying-up or low temperature, which causes this appearance. When proper conditions are restored, normality of form may be resumed.

I have seen about half a dozen stained specimens which I interpret as multiple fission. This is a small number out of the hundreds of specimens passed in review, and yet multiple fission is probably a rare process, which does not take place in the mouth cavity. The suspected forms have no protective walls, have usually been found in close contact with leukocytes or epithelial cells, and are somewhat irregular in outline (Fig. *k*).

There is no limited number of merozoites as eight or four in the *E. coli* and *E. histolytica* cysts, but the number may vary from eight or nine to more than a dozen. Those I have seen do not seem comparable to the reproductive cysts of *E. coli* and *E. histolytica,* but suggest rather the merozoite formation in Plasmodium, and probably serve merely to spread the infection in the host.

Endamoeba buccalis follows the course of all protozoa in encysting. It first becomes spherical and inactive and begins to diminish in size. I induced a kind of encystment once by leaving *E. buccalis* sealed on a slide for six hours with the temperature gradually going down. These did not in that time change much in size, but the food vacuoles paled and seemed to dissolve in the cytoplasm.

Normally, encysted forms are from one-half to two-thirds the size of the active trophozoite; they usually show some faint, rounded inclusions, probably the remains of food vacuoles, and a clear wall slightly spaced from the animal protoplasm proper (Fig. *l*). Encystment seems to be for protection against adverse conditions rather than for multiplication, as is shown by the following observations.

RELATION OF HOST AND PARASITE

Encystment with this form is not as rare as Craig (1916) believes. One strain followed daily for months will show the encysted condition from time to time, and my records show that encystment in *Endamoeba*

buccalis is closely connected with the "scarce periods." As stated previously, daily examinations showed numerous active endamoeba in the scrapings from a tooth for ten and fourteen consecutive days. Then would intervene two to four days when few could be found, though the same region was carefully explored. Such forms as may be found at these scarce times are recorded as "sluggish," "spherical," "encysted." Most of the active forms left were small. Very deep probings into the gums around the tooth sometimes procured a few larger ones, tho finally even these would fail.

Often when the parasites became numerous again they were sluggish and half encysted (Craig's precystic stage) for a day; then they became active and flourished again as usual for two or more weeks. Once they did not disappear for four consecutive weeks, but at the end of that time they were gone completely.

Now what is the explanation of this periodic appearance and disappearance? I laid it at first to mouth-cleaning, until that was carefully tested out. I then considered that changes in the host might account for it, and following out that clue I found by my journal that practically every period of scarcity was accompanied by a time of low vitality on the part of the host as manifested by some slight indisposition. Indigestion accompanied at least three of these disappearances. I concluded that physiological changes of the body, whether normal or abnormal to the host, change the chemical reaction of the body fluids, notably the saliva in this case. These changes may be too slight even to be analyzed, and yet they affect the very sensitive endamoeba living in that medium. As in the case of free-living amoebae, encystment follows when conditions change from the normal, so with *buccalis*, when the saliva changes in its chemical qualities, the parasite encysts either partially or wholly. It withdraws into the periosteum, however, for this purpose.

Further evidence for this was furnished by extracting the infected tooth during a scarce period. The endamoebae had flourished for four weeks and then disappeared; for two days none had been found. The tooth showed an ulcer at the root, and by aid of the binocular many lesions as described by Bass and Johns (1915) were seen on the periosteum. These lesions examined microscopically showed many leukocytes and few amoebae, but on the periosteum around them, and especially where it joined the tooth, amoebae were extremely numerous. These seemed to be in very close contact with the tissue, tho some were removable by means of a fine scalpel. All of the endamoebae thus found were in partial or complete encystment.

I think this withdrawal of *Endamoeba buccalis* to the gums to encyst explains why encystment has been so seldom reported. I believe further that the end of the reproductive phase, binary fission, occurs

normally either in the gum tissue or in close contact with it; for although spindles are frequently seen in smear preparations, actual division of the cytoplasm has never been seen. Since the gums of infected patients cannot be sectioned, I now have under way a study of the tonsils in the hope of throwing light on the intracellular phase of the life-history. An understanding of the periods of disappearance of *E buccalis* would be valuable in the treatment of pyorrhea if it be caused by this parasite.

TONSIL STUDIES

With a view to determining whether *Endamoeba buccalis* is ever a tissue dweller, I have studied the surface scraping of tonsils both before removal from the patient and after. In only one case did I find endamoeba in the fresh smear, and their detection is certain if they occur. In no case have I been able to identify parasites in the paraffin sections. In view of the fact that Smith, Middleton, and Barrett (1914) found amoebae so plentifully on the tonsils examined, I have been surprised to find none in any of my examinations. If *E. baccalis* is a tonsil parasite, it seems very probable that it penetrates the tissue of so soft an organ and undergoes some interesting stages of development which I believe are constantly taking place in the gums, but which I have no means of demonstrating.

As my work in this direction is scarcely begun, the negative results thus far are by no means conclusive or even discouraging.

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ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

II. ADDITIONAL OBSERVATIONS UPON MYXOBOLUS MUSCULI OF FUNDULUS AND A NEARLY RELATED SPECIES, M. PLEURONECTIDAE OF PSEUDOPLEURONECTES AMERICANUS

C. W. Hahn

Reference to the multiplicative stages of this parasite was made in a former paper (Hahn, 1913). At that time the true parasitic nature of the trophoblasts of both the multiplicative and propagative stages was insufficiently established. The relative virulence of the protozoon and the bacteria also needed further confirmation. Subsequent studies leave no doubt as to either of these points.

In almost every diseased integument, gill, or flesh wound which one examines, there will be found among the decadent tissues a few or many clear, white, even-contoured bodies which rarely take up any stain, no matter what treatment the tissues may be subjected to. The bodies are therefore in strong contrast with the surrounding tissues. If conditions are such that the parasites can be seen at all, the tissues must have taken up more or less of the stain. It was hoped that by using a variety of stains in different combinations with a wide range of fixatives, one might succeed in finding a treatment that would reveal the nucleus and perhaps other cytoplasmic contents of the parasites. No very encouraging results were obtained with the reagents that follow.

After fixation with alcohol (Abs. 62 per cent), ether (32 per cent) and 40 per cent formaldehyd (6 per cent), I used Giemsa, toluidin blue, methylene blue, thionin, Bismarck brown, fuchsin, anilin blue, Bordeaux red, neutral red, dahlia violet, sudan III, indigo carmin, methyl violet, alizarin, rose anilin violet, carbol fuchsin, picro-nigrosin, safranin, and hematein combinations. With corrosive sublimate solutions in different solvents and after chromic, chromosmic, and many other common and some unusual fixatives, such as tannic, malic and formic acids, the following stains were employed: Ehrlich's hematoxylin, Mayer's hematein, safranin, fuchsin, Heidenhain's hematoxylin, and brazilin. Both Mayer's hematein and Heidenhain's hematoxylin give to the cytoplasm of the parasite a slight clouded effect which renders it visible throughout. Rarely a medium or large-sized trophoblast has a faint blue nucleus, and less frequently a small dense spherical nucleus. Brazilin has given promising results when used in connection with a 5 per cent aqueous chromic acid fixation.

The trophic stages of the multiplicative cycle are much more frequently encountered in all the tissues I have examined. They also occur in much larger numbers, especially the minute stages. Thousands of them are frequently distributed more or less equally throughout the myoplasm of certain areas of muscle fibers (Fig. 9). A few are interfibrillar. Such muscle fibers may or may not give evidence of hypertrophy. The size of the parasites in one and the same tissue may vary from 1.5μ to 80 or 90μ in diameter. A very good picture of them has already been published in Figure 12, Plate XX, of the paper mentioned above. The trophic stages of *Chloromyxum clupeidae* (Fig. 8) appear to be very nearly the same in appearance as those of *M. musculi.*

In shape the multiplicative trophoplasts are circular or oval when small. Older ones have slight blunt extensions here and there over the surface. Occasionally a long pseudopod is encountered. Since these observations are made from fixed smears, it is probable that in life the display of activity on the part of the pseudopods would be very striking, could it be seen. As yet I have observed no striking activity in numerous fresh tissues. In very large parasites the cytoplasm is finely granular. The smaller ones appear to be structureless. Trophoplasts of moderate size frequently have a thin border of stainable material covering a part or all of the surface. This suggests an excretion or surface deposit, but is in reality what remains of the muscle nucleus which has been atrophied under the action of the parasite (Fig. 4). This can be demonstrated by the study of a large number of cases, when it will be found that there is a complete series of stages between the condition here described and normal nuclei.

Multiplicative trophoblasts have been found in muscle epidermis, gill epithelium, and connective tissue. All of these tissues are attacked and undergo cellular degeneration. The nuclei and mucous cells usually remain in various stages of hypertrophy and constitute a very misleading series of artifacts.

The staining reaction and the general appearance of the multiplicative trophoplasts are such as to suggest strongly that these bodies are some fatty or lipoid degeneration product. After many months of doubt, preceded by many more during which they were overlooked because it was assumed that the bodies in question were oil globules, it finally proved impossible to exclude them from the myxosporidian life-history. Authority may be found in the literature in support of both interpretations. It is an accepted fact (Adami, 1910) that with the hypertrophy of muscle, uniformly distributed fat bodies are to be expected. It has also been shown that the hypertrophy of the nucleus sets up changes in its immediate vicinity that result in lipoid substances.

The association of hypertrophied nuclei and Myxosporidia described above fits these specifications very well. On the other hand, small globular bodies within and between the muscle bundles were taken by Pfeiffer (1891 : 106) to be germs of a myxosporidian.

The evidence upon which I have based my decision is (1) the failure of either osmic acid or sudan III to give a fat reaction, whereas oil globules on the same slides give a typical reaction. For the sudan III tests the tissues were fixed in aqueous formaldehyd solution, treated with a low-grade alcoholic solution of sudan III, and preserved in glycerin. (2) The large trophoplasts show granular cytoplasm and a faint nucleus at times, when stained with Mayer's hematein and Heidenhain's hematoxylin. (3) The trophoplasts occur in graded sizes as if belonging to the same stage of growth. (4) Many trophoplasts have pseudopodial extensions that have a strong motile suggestion. (5) Many muscle fibers in an advanced stage of hypertrophy are free from the bodies in question; they have migrated or operated in some other part. The products of degeneration would be expected to be uniformly distributed in all atrophied muscle fibers. (6) The sporoblasts of both *M. musculi* and *Chloromyxum clupeidae* have exactly the same oil-like appearance as the multiplicative trophoplasts and reactions, but contain some characteristic body that belongs to the sporogenesis, such as the myxospore itself (Chloromyxum) or the sporoblast nuclei. (7) When one compares the trophic stages of the multiplicative cycle with the propagative cycle of the Myxobolus or both with similar stages of the Chloromyxum, four kinds of bodies may be recognized. If the structures in question are artifacts, this distinction into two classes would not conform exactly to the conditions required by the protozoon life cycle as to equality of development of all individuals present. This is exactly what is found in regard to both of the genera here described. Either all the parasites are young trophoblasts of the multiplicative cycle, or all are in some phase of the propagative cycle. (8) Many of my preparations have been treated with ether and absolute alcohol. Oils are extracted by this treatment. Yet most of the structures in question show some evidence of a solid content, whereas casts of fat bodies, when encountered, are clear and structureless.

Many observers have found in fresh tissues small motile, structureless bodies, and also cells with nuclei, which they have assumed were parasites. I have examined fresh infected tissues of both the herring and Fundulus. While able to recognize the trophoplasts and sporoblasts, it has never been possible to be certain that the suspected objects were parasites until they were either connected by stages to sporocysts containing myxospores or until they had been verified in fixed and stained preparations. Pathological tissues frequently con-

tain artifacts resulting from the products of degeneration (Hahn, 1913: 197) which are very misleading. There are also numerous tissue cells and ameboid cells with well-developed pseudopods in atrophied tissue, especially in the epidermis of such fish as the flounder. Under these circumstances, one is inclined to place little confidence in observations based upon fresh tissue alone. It is probable that the observations of Pfeiffer (1893 and 1891), Thélohan (1893), and Megnin on the trophic stages of *M. pfeifferi* were correct, but one must always feel doubtful about the reliability of one's interpretations when good and sufficient reasons for considering any fresh cell as a parasite are not given.

The multiplicative trophoplast continues to grow in size until it is over 50μ in diameter. Although not observed alive, the shapes and general appearance lead to the conclusion that they are motile. They usually occur singly in comparatively uninfected portions of the tissue. In shape they vary from a long gregarine-like structure with very finely granular endoplasm and a shallow clear cytoplasm, to a smooth oval or circular mass when seen in profile. These large individuals may reach a diameter of 50μ (Fig. 14). Associated with them in the same tissues one rarely finds schizonts containing minute spores. The schizonts range in size from 40 to 55μ. These bodies are embedded in the muscle fiber in a cavity which they completely fill, giving precisely the same appearance as the large immature schizonts (Fig. 12). The multiplicative spores within are about 1.5μ in diameter. In the few cases which I have examined, they have not taken up the stain, but are visible owing to the presence of a residual material which retains a moderately intense stain. Free multiplicative spores are common, and like all multiplicative stages, they are also characterized by the non-staining quality. Occasional schizonts containing spores are encountered in fresh tissues. They have also been seen and distinguished from propagative stages in sections. As yet none of the latter were so large as those here figured. The scarcity of sporulating schizonts is no doubt to be attributed to the rapidity of the dissemination of the spores under the muscular activity. As previously noted, the schizonts have already migrated into fresh tissues by the time they have reached any considerable size.

One might suspect that the bodies produced by the so-called schizogony and figured here are bacteria. Very similar colonies of bacilli have been described elsewhere (Hahn, 1913). These bacilli do not occur in muscle which is essentially normal, and they are not accompanied by interstitial material when isolated and embedded in the myoplasm. The individual here figured was fixed with Flemming's fluid and stained with safranin. This combination cannot be expected to stain bacteria. Failure to stain with Giemsa and methylene

blue does occur in certain bacilli which are common in the necrotic region of these sores. The stain is therefore not so reliable a criterion as location, uniformity of size, association with other free individuals, etc.

The time required for one complete multiplicative cycle is approximated in the discussion of inoculation experiments.

Schizogony in *M. musculi* would be expected if the schizonts containing spores had not been seen, since the peculiar distribution of the trophoplasts cannot be readily explained by any other kind of multiplication. The smallest individuals are usually very numerous in localized regions and differ but little in size. Older stages occur in fewer and fewer numbers unaccompanied by the small forms, proving that they have migrated from the focus of the multiplicative process.

Multiplicative reproduction in Myxosporidia was demonstrated by Cohn (1896) in *M. lieberkuhni* and by Doflein (1898) in *Glugea lophii.* Minchin (1903) describes fission and budding and refers to the multiplicative schizogony of *Glugea lophii* as a kind of schizogony, adding that "this kind of reproduction is probably very common, if not universal, in the tissue and cell-infecting Myxobolidae and Glugeidae." Doflein (1911) makes provision in an outline of the life-cycle of a typical myxosporidian for schizogony and suggests it is typical as a preliminary to sporogenesis, but gives no specific illustrations and does not elaborate this as a process of multiplicative reproduction independent of the formation of sporoblasts.

Laveran and Mesnil (1902) review the various methods of reproduction in Myxosporidia, referring to budding as described by Cohn in *Myxidium lieberkuhni,* also to binary fission as described by Doflein in *Chloromyxum leydigi,* and to the simultaneous division of the nuclei in the process of plasmotomie, but cite no typical cases of schizogony.

The occurrence of multiplicative schizogony in a species of Myxobolus in the bile of the flounder has been observed by the writer. Plehn (1905) figures and describes a schizont with a large number of multiplicative spores in *Lentospora cerebralis* from the salmon. It causes the so-called "twist disease" (drehkrankheit). He supposes that the spores develop into a cell which has a conspicuous nucleus that is lacking in the spores. In view of what has been learned about *M. musculi,* it seems more probable that Plehn's nucleated cells are in the line of the propagative cycle. They are probably sporoblasts or gametoblasts. The non-nucleated spores are perhaps multiplicative spores.

A schizont with ten multiplicative spores has been described by Nemeczek (1911) in *Henneguya gigantea.*

It is now certain that the propagative cycle starts with a spore which is unlike the meront of the multiplicative stage. Beginning with

the spore, the staining properties of the propagative trophoplast are distinctly different. In the paper already cited, Figure 14, Plate XX, represents a schizont with differentiated spores. They are probably not multiplicative spores as there stated. The latter are smaller and their nuclei do not stain. The propagative spores occur free in the myoplasm in fewer numbers, but with about the same pathological effects and habits as the multiplicative spores. Because of the intimate and constant association of the small propagative spores having nuclei with large ameboid trophoplasts (Fig. 14), I have concluded that the former develop into the latter. This view might be less tenable if there was not a sharp limitation to the range of development which the parasites have attained in any given tissue.

The fate of large propagative trophoplasts such as are shown in Figure 14 is probably some form of multiplication which results in moderate-sized sporoblasts. It is possible that they develop directly into primary sporoblasts, such as are undoubtedly represented in a trophic condition in Figure 5, and in a quiescent condition in Figures 6 and 7. Such an interpretation conforms to the accepted life-history for other species of Myxobolus. But if one is right in supposing that certain elongated spores of moderate size which have been occasionally encountered isolated (Hahn, 1913: 113, Figs. 17 and 19, Plate XXI), and in small sporogenic cells (Ibid.: Fig. 16, Plate XX), are to be included in the life cycle of *M. musculi,* then it is difficult to reconcile the stages represented with the sporogenesis as hitherto described by Mercier (1908), Keysselitz (1908), Schröder (1907, 1910), and others. There are apparently three different kinds of spores in *M. musculi.* One belongs to the multiplicative cycle and may without doubt be called an asexual type. The other two are very probably to be associated with the propagative cycle, and one may expect that they have some sexual significance. Of these, one is a spherical spore 2.5 to 3μ in diameter, which has a small well-defined nucleus and faintly staining protoplasm. They occur in large schizont cysts (Hahn, 1913, Fig. 14, Plate XX), and are produced in rather large numbers. They no doubt become the sporoblasts that are so numerous in tissue adjacent to them in the one tissue where they have been encountered. The latter are identical to sporoblasts such as are figured in this paper (Figs. 5, 6, and 7). The other type of propagative spore was encountered in the same slide as the above and in the immediate vicinity of them. They are contained in cells having a diameter of about 12μ. These sporocyte cells appear to be of independent origin. They occupy the space left by an atrophied muscle fiber. The contained spores are 2.5 by 4μ in size and have rather large nuclei. Each sporocyte contains from four to twelve spores.

The elongated type of spore not yet has been satisfactorily explained. If the spherical spores which contain a stainable nucleus are identical with what was assumed to be multiplicative spores, the elongated spores may prove to be sporocytes. I am not altogether certain that the one tissue represented was not harboring a double infection. A third hypothesis is that the small spherical spore is a microgamete and the larger elongated spore is a macrogamete. In this connection it is interesting to note that in the muscle fibers where typical medium-sized sporoblasts are abundant, occur also several small elongated cells with pointed, densely staining nuclei, having a terminal position (Hahn, 1913, Fig. 18, Plate XXI). One may suppose that these are motile microgametes, but at present no evidence is available to substantiate the hypothesis.

One may conclude with reasonable assurance that the sporoblasts do arise from a very common type of spore which arises by a process of schizogony, and that the propagative sporoblasts are sufficiently differentiated from the multiplicative spores to be easily distinguished while yet in the schizont cyst. I believe that after a succession of multiplicative cycles ending in multiplication by schizogony, there follows a schizogony which generates spores that become differentiated very early into either gametes or primary sporoblasts. (See also page 102 in the first section of this paper for time relations.)

The primary generative cells of *M. musculi* certainly do not arise by free cell formation in large myxoplasms, such as is the case in *M. pfeifferi* of the barbel, and *Sphaeromyxa labrazesi* (Lav. and Mesnil), according to Schröder, 1907. The primary propagative cells of *M. musculi,* on the other hand, are set free simultaneously by one or the other of the schizonts described above. This conclusion is based not only upon the existence of two or more types of schizonts, but upon the fact that in four tissues where sporoblast stages occur, they are very numerous and at approximately the same state of development.

The propagative stages have not been encountered so frequently as the multiplicative stages. This is probably due to the fact that they are not nearly so abundant. In some tissues one may find both kinds present, but according to my observations, one or the other is always greatly predominant. With the exception of the elongated spores which occur in certain small cysts that have been figured and described elsewhere (Hahn, 1913: 204, Fig. 16, Plate XX), there is no evidence that the multiplicative and propagative trophoplasts do not have practically the same structure and appearance when small.

There is absolutely no evidence that they are generated consecutively by budding or fission or plasmotomie, but quite the contrary. The propagative stages gradually differentiate from the multiplicative type, and by the time one can positively identify them as such, they are dif-

ferent both in appearance and staining reaction. When unmodified by the contraction of the muscle fibers, they are more or less spherical bodies with almost transparent glassy cytoplasm and a small vaguely staining nucleus (Hahn, 1913). Older conditions are shown in Figure 18 of the paper just referred to. They have a large well-stained nucleus and fit loosely in the space which they have eaten in the myoplasm. The shape varies from round to oval, and evidence of active mobility or of pseudopods is often lacking. Somewhat earlier stages, when compressed by the shortening of the muscle fiber, have long extensions of the cytoplasm (Fig. 5). The nucleus is also extended into a long slender mass and sometimes extends into the thicker portions of the protoplasmic branches. This condition does not seem to be quite normal. Many cases of less compressed myxoplasm occur as regularly distributed spindles.

Besides very small sporoblasts, there are numerous good examples of larger sporoblasts and sporocysts in all stages of sporogenesis and sporocysts with immature and more or less mature myxospores. Stages not figured in the plate of this paper will be found in my paper of 1913.

When unmodified by the contraction of the muscle fibers, the sporoblasts are probably more or less spherical with a small nucleus (Fig. 7), or a large one (Fig. 6), and almost transparent vitreous cytoplasm. The nucleus does not stain intensely, but is more or less free from characteristic stainable bodies (Hahn, 1913, Figs. 18, 21 and 35, Plate XXI). Presumably these are the same stage of the organism as those which are encountered frequently in an ameboid condition fitting loosely into irregular transverse clefts of hypertrophied muscle fibers (Fig. 5). The conditions in some cases, such as Figures 6 and 7 here and Figure 18 (Hahn, 1913), suggest that there is an advanced condition in which ameboid activity is lost. If so it is probably just preceding the process of sporogenesis. There is a transition between the ameboid condition and the inactive condition wherein the myoplasm is divided into narrow transverse partitions by very numerous spindle-shaped cells which lie with their long axis at right angles to the length of the fiber. It is unsafe to say to just what extent the mechanical action of the muscle and the number of parasites are responsible for these alterations in shape. Thélohan (1891) figures and describes exactly the same condition in fish muscle fibers. He also interprets them as sporogenic cells.

Sporoblasts are sometimes so closely packed in the space once occupied by a muscle fiber that, though the form of the fiber remains, the myoplasm can be seen only rarely (Fig. 18). When thus packed together, these cells form a pseudo-epithelium which can be distinguished from a slightly degenerated epidermal or mucous epithelium with the greatest difficulty. Practically one must depend in many

cases upon a general resemblance to other epithelial masses in the same tissue, the cells of which have entered upon some easily recognizable stage of sporogenesis. Such pseudo-tissues are either more or less obscured by the hypertrophied myoplasm, muscle, and vascular nuclei, or are so closely packed that unless spread out mechanically in smear preparations, suitable specimens for drawings cannot be found. It is such a scattered group that was selected for the camera drawings represented in Figures 7 and 18. For purposes of reproduction it was necessary to exaggerate the detail of both nuclei and the cytoplasm of the parasites. The disinclination to stain is still retained to a limited degree in the propagative stages.

The epithelioid tissue just referred to must not be confused with another condition which has already been described (Hahn, 1913), in which the hypertrophied nuclei of vascular and connective tissue occupy the mold of a muscle fiber and, mingled with the remnants of the myoplasm, resemble a bit of degenerating epithelium.

The identity of the cells of which these pseudo-tissues are composed rests upon very positive evidence. Not only can one easily find obvious differences between them and true epithelium, but there are many such masses lying among the atrophied muscle fibers, many of which are in stages of sporogenesis like that represented here (Fig. 3). On a single slide one cannot fail to connect stages identical to those of Figures 3 and 6 (below) with the less obvious stages in Figures 7 and 18. There are also interesting isolated groups of sporoblasts identical in appearance to those forming the epithelioid masses that occupy small spaces in the myoplasm (Fig. 6). Differences in the size of the nuclei are to be expected when it is recalled that we are comparing primary and secondary sporoblasts with pansporoblasts and possibly other stages of the propagative cycle. Figure 7 is magnified 560 diameters and Figure 6, 750 diameters. It is noteworthy that the group in Figure 6 is accompanied in the same fiber by a pansporoblast with ten or eleven nuclei. Between the former and the latter the hypertrophied myoplasm has lost the fibrillae. That the bodies represented in this fragment of muscle fiber were invading parasites is clearly obvious. The muscle hypertrophy alone is significant. Adjacent fibers have numerous isolated parasites, while the epithelioid masses and numerous stages of sporogenesis like Figure 3 are on the same slide from which the group in Figure 6 are taken.

It is rather by analogy with other Myxobolus than by direct observation that one must interpret the various propagative stages which have been encountered in the tissues of Fundulus. The majority of the older stages such as those in the pseudo-epithelium are probably sporoblasts. As already stated, those with large and small nuclei may prossibly be gametoblasts. There are some very large spherical stages

with two large and two small nuclei from which the sporogenesis starts. With numerous succeeding stages leading up to Figure 3 one has, at least, ample proof that trophoblasts whose nuclei stain are destined to give rise to propagative spores, i. e., myxospores.

It is of considerable interest that the early propagative stages like trophoplasts have a destructive career. Their scattered distribution in the younger stages is due to a rather extensive motility either upon the part of the parental schizont or upon their own activity. But when nearly mature they evidently become less active. The masses which occupy the mold of the muscle fibers suggest in a general way pseudocyst formation such as has been found in the gill (Textfigs. 1, 2, and 3), and is common in many of the other species (*M. pfeifferi* of the barbel disease).

The pseudo-epithelium (Fig. 18) formed by the propagative stages of *M. musculi* is a most remarkable condition and deserving of more attention. The simulation of normal or slightly hypertrophied host tissues is a most deceiving circumstance. When a parasite having such qualities occurs in small numbers and more or less isolated, the most careful observer will fail to recognize it. Moreover, if a sufficient number of tissues is not available, suitable stages for a positive identification will be wanting. The facts just noted are important because of their possible bearing upon the epithelioid tissues of mammalian cancer. Adami (1910) states that cancer tissue resembles nothing so much as a parasite upon the mammalian tissues. The propagative stages of *M. musculi* frequently give the appearance of a typical epithelioma.

SUMMARY

For the results of inoculation experiments bearing upon the life-history see the summary at the end of the first section of this paper.

1. *M. musculi* has a series of multiplicative cycles starting with the myxospore, followed by a propagative cycle, ending in the myxospore.

2. There are two or more types of schizonts and schizogony.

3. Multiplicative reproduction is carried out by means of a large schizont which gives rise to a very numerous progeny of very minute spores.

4. The multiplicative spores and trophic stages do not take up any stain thus far utilized, with one not very satisfactory exception.

5. Multiplicative trophoplasts and schizonts migrate into uninfected tissue, particularly just before the quiescent period preceding schizogony.

6. All propagative stages possess a nucleus which reacts to basic stains.

7. The schizonts which give rise to primary propagative spores also migrate into new tissues before undergoing schizogony.

8. Another process of schizogony exists in which the schizont is very large and the spores, though larger than multiplicative spores, are small and have a small nucleus which reacts to a basic stain.

9. A third type of propagative schizogony may possibly exist in which the schizont is small and the spore very large, with a large nucleus which reacts to a basic stain.

10. If the conditions in 8 and 9 are trustworthy, there is a differentiation of gametes into macro- and microspores.

11. Sporoblasts, whether arising from conjugation or destined to conjugate, are ameboid, trophic, having the ability to migrate to a limited extent only when immature, and losing this property later.

12. Multiplicative stages perforate muscle fibers extensively and bring about profound hypertrophy. Propagative stages while yet trophic are also predacious, but to a less degree. The latter give rise to characteristic irregular transverse clefts in the fibers. Such clefts vary in number, shape and size, and occur in more or less atrophied fibers only.

13. The passive propagative sporocytes pass through all the characteristic stages of sporogenesis such as have been described for *M. pfeifferi* (Keysselitz, 1908).

14. Closely packed primary and secondary sporoblasts form an epithelioid tissue which at times has the appearance of integumentary epithelium and closely resembles mammalian epithelioma.

15. Pseudocysts occur, having many myxospores in a common sporocyst plasm. They probably arise by the fusion of closely packed sporocysts.

MYXOBOLUS PLEURONECTIDAE OF WINTER FLOUNDER

A winter flounder (*Pseudopleuronectes americanus*) having open sores was collected by Dr. W. E. Sullivan in the vicinity of Woods Hole. When examined, the flesh proved to have undergone pathological changes almost identical to what has already been described in Fundulus. The flounder was 8 inches long and had three lesions. One on the dorsal side was ¾ inch wide and 1 inch long; the other two were smaller. The integument was either white and partially decomposed or completely gone. The underlying flesh was red and vascular at the surface and less transparent than normal. These external conditions resemble the appearance of the myxosporidian disease of Fundulus as much as one could expect, considering the difference in the integument, skin, and color of the flesh of these fish.

Suitably stained smear preparations of the flesh present almost the same pathological conditions as are found in the fundulus disease.

There are present hypertrophied muscle fibers and epithelium cells, degenerated nuclei, mucus cells, and numerous bacteria limited to the most disintegrated parts. Numerous fibers contain considerable numbers of unmistakable trophic stages of the multiplicative cycle of a Myxobolus. One could not distinguish these from the same stages of *M. musculi* of the Fundulus. Large multiplicative schizonts, almost mature sporoblasts, and myxospores are also to be found in the same tissues. With the exception of the myxospores, there is no noticeable difference in the propagative stages and those of *M. musculi.*

The myxospores are not very abundant, but they are suitably stained for comparison with other species. One has no difficulty in distinguishing them from the myxospores of *M. musculi* by their shape (Fig. 2). The latter are tapered more at the polar end and the polar capsules are drawn out into a narrow apex.

The flounder parasite has myxospores which are 14.8μ long and 11.9μ wide. Those of *M. musculi* are 14.3μ long and 6.7μ wide. Immature myxospores of *M. musculi* are 12 by 7.5μ by actual measurement. In both cases the figures here given are the average of several different spores. The flounder myxospore has polar capsules which are 6μ long by 3.7μ thick, and the fundulus parasite has polar capsules 6.5 by 2.0μ. The flounder myxospore is therefore 2μ shorter than *M. pfeifferi* and 1.9μ narrower. In shape and appearance it resembles the latter closely. Allowing for slight variations in size and shape due to difference in maturity, the discrepancy between the myxospores of the two fish is too great to consider them as belonging to the same species. The inoculation of one host species by myxospores from the other will easily settle this question. In the meantime, the name *M. pleuronectidae* is proposed for the flounder parasite. It is probable that many species of the flatfish are subject to attacks by this parasite.

It is interesting to note that one Chloromyxum myxospore was encountered in the tissues of this flounder.

The articles cited in this portion of the paper will be listed at the conclusion of the paper in the September number of the JOURNAL.

Explanation of Plate

Fig. 1.—Five sporoblasts of *C. clupeidae* from the same slide as Figure 16. Note the unstained cytoplasm of the sporoblasts with thin filaments of host tissue residues separating one sporoblast from another. Compare this non-staining material with that in Figures 8, 11 and 16. Note the square form of both myxospores and sporoblasts. The capsule nuclei are applied to the polar capsules in the right hand lower sporoblast. The sporoplasm is unstained. The sporoblasts each contain a developing spore the nuclei of which are probably imperfectly stained. × 1575.

Fig. 2.—A myxospore of *M. pleuronectidae* from a lesion of the back of a winter flounder (*Pseudopleuronectes americanus*). ×1575.

Fig. 3.—A sporoblast of *M. musculi* undergoing sporogenesis. The specimen here represented is one of many in a mass of cells similar to that in Figure 7. The dark border is stained serum. A space exists between the latter and the sporoblast, due to shrinkage. There are about 30 well defined nuclei. A few appear to be elongated as if about to divide. ×560.

Fig. 4.—Three trophoplasts of *M. musculi* from the eye muscles of an inoculated Fundulus that had died from a general infection of the head region. The parasite has not taken up any stain while the host tissue has. These three cases show as many stages in the hypertrophy of muscle nuclei which the parasites have apparently attacked. Note the small trophoplast is associated with a nucleus showing normal alveoli while the larger trophoplasts are associated with nuclei from which alveoli have partially or completely disappeared. ×1575.

Fig. 5.—A fragment of an atrophied muscle fiber from a large open lesion of Fundulus containing propagative trophoplasts, possibly sporoblasts of *M. musculi*. I regard these as earlier than in Figure 6. The position of the long axis of the sporoblasts and their cavities, which is at right angles to the length of the muscle fiber, is due most likely to the contraction of the fiber. Compare the granular nuclei in this with Figures 6 and 7. ×300.

Fig. 6.—A muscle fiber from Fundulus with several sporoblasts of *M. musculi* in the same cavity and one isolated. The many small nuclei of the latter indicate that it is in an advanced stage of sporogenesis. The large size of the nuclei in the others indicates that they are in a much later condition than those shown in Figures 5 and 7. Atrophy of the myoplasm is just beginning. ×750.

Fig. 7.—A group of sporoblasts of *M. musculi* at about the same stage of development as in Figure 5. The group lies adjacent to an epithelioid tissue which has replaced a completely atrophied muscle fiber. These cells are drawn out of the mass sufficiently to permit drawing details which are not possible in the compact masses, one of which is shown in Figure 18. ×560.

Fig. 8.—Portions of four muscle fibers from the dorso-branchial region (not body muscle) of the young of *Clupea harengus* which had numerous pseudocysts of myxospores of *C. clupeidae*. No myxospores occur in the head region. All the muscle is thus riddled with the trophoplasts of the Chloromyxum. They are both inter- and intra-fibrillar. When intercellular, note they have crowded the muscle fibers. Fibrillation and striation of the muscle fibers is entirely lacking. ×300.

Fig. 9.—A portion of a muscle fiber from the body muscle of *F. heteroclitus* having a typical infection of very young multiplicative trophoplasts of *M. musculi*. ×300.

Fig. 10.—Three mature myxospores of *C. clupeidae* showing the four polar capsules stained. ×1650.

Fig. 11.—Sporocyst of *C. clupeidae* in an atrophied myoplasm from anterodorsal body of muscle of young *C. harengus*. Compare with Figures 1 and 16. Note the increase in size. Sporocyst plasm is unstained. Sporoplasm has assumed a more or less rectangular form. ×1575.

Fig. 12.—A multiplicative schizont of *M. musculi* in the myoplasm of an atrophied fiber from Fundulus. ×750.

Fig. 13.—A medium sized trophoplast of *C. clupeidae* migrating from an old tissue to new. ×300.

Fig. 14.—A large schizont of *M. musculi* from the same slide as Figure 12 which has not yet undergone schizogony. ×560.

Fig. 15.—A large schizont of *C. clupeidae* from the body muscle of young *Clupea harengus* in which no pseudocysts are present and no myxospores were found. These schizonts are abundant in comparatively normal muscle fibers. ×300.

Fig. 16.—Four sporoblasts of *C. clupeidae* from inflamed body muscle in the ventrolateral region. The two left-hand sporoblasts are enclosed in the sporocyst and the right-handed sporoblasts are free. The latter are comparable to the shaded portions in Figures 1 and 10. ×1650.

Fig. 17.—Photograph of a typical lesion in *F. heteroclitus* which afterwards proved to be caused by a typical infection of *M. musculi*. Note the swelling, the loosened and projecting scales, and the open central area from which the integument has disappeared.

Fig. 18.—A mass of sporoblasts of *M. musculi* giving the appearance of an epithelium. The truncated form of the mass is due to the fact that these sporoblasts have occupied the space left by the muscle fiber whose hypertrophy they have brought about. ×300.

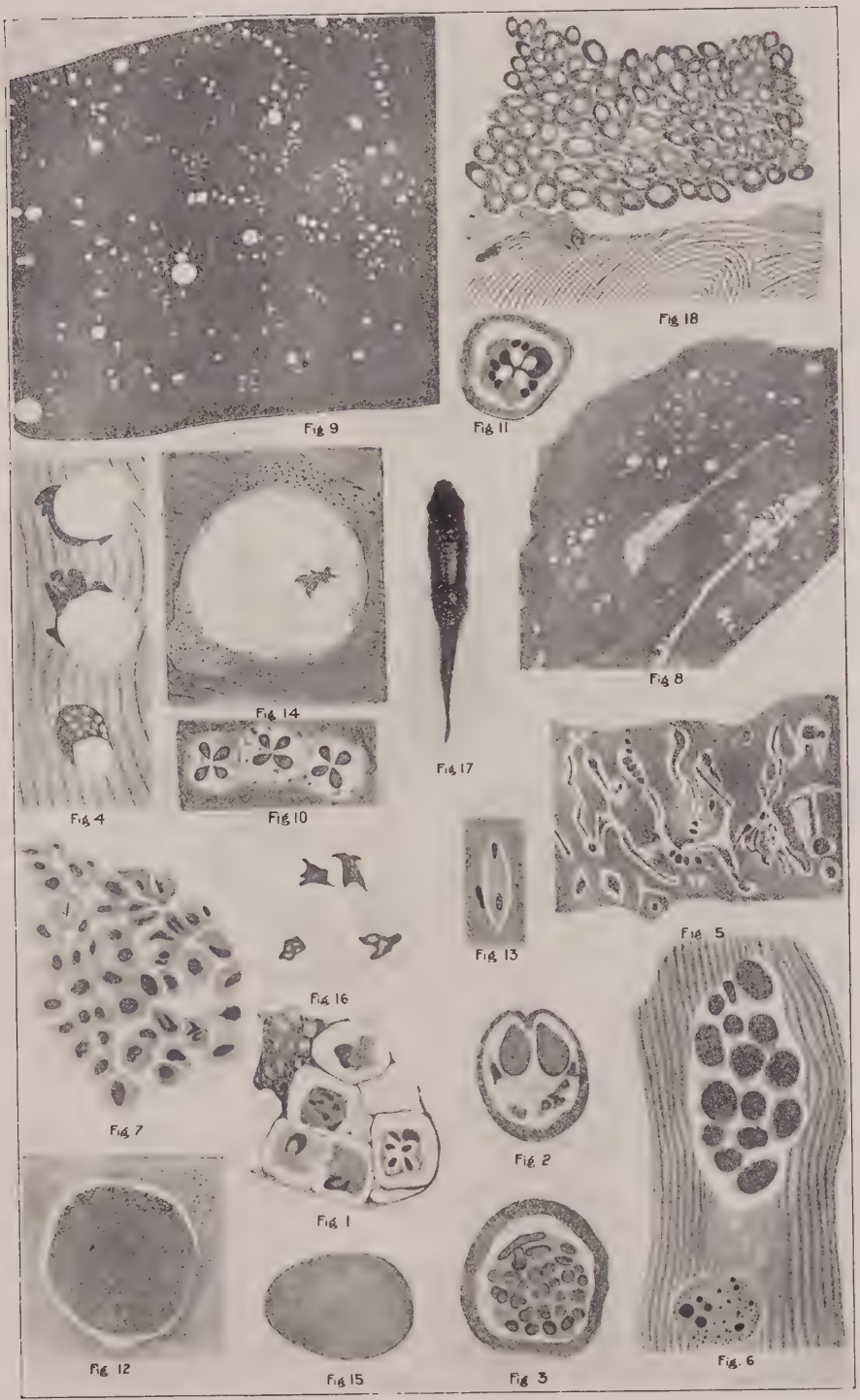
Fig. 9
Fig. 18
Fig. 11
Fig. 8
Fig. 14
Fig. 17
Fig. 4
Fig. 10
Fig. 5
Fig. 13
Fig. 16
Fig. 7
Fig. 2
Fig. 1
Fig. 6
Fig. 12
Fig. 15
Fig. 3

CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. II.*

MYXOBOLUS TOYAMAI NOV. SPEC., A NEW MYXOSPORIDIAN PARASITE IN CYPRINUS CARPIO L.

ROKUSABURO KUDO

While studying Cnidosporidia in some fresh-water fishes during the last few months, my attention was attracted to a minute white spot on the branchial lamella of a *Cyprinus carpio*. Examination under the microscope showed that the white spot was no other than a round cyst of a myxosporidian containing numbers of ripe spores each having only one polar capsule. The fish that harbored the Myxosporidia was a year old, having a length of about 6 cm. On searching carefully all the branchiae of the fish under the dissecting microscope, I found another round body situated near the free end of a branchial lamella, the diameter of which was about 200μ. Since that time, many fishes of the same kind, and reared in the same pond where the above-mentioned infected fish had been found, have been examined for the same parasite, but it has not been found again. Consequently, the material is too scanty for detailed study. I will try, however, in the following pages, to give the results of observations on the one fish, which are probably of some interest, since the morphology, and especially the life-history, of the unicapsulated Myxosporidia seem, so far as I am aware, to have been left in obscurity.

The branchiae of the infected fish were cut into pieces, fixed with Schaudinn's or Fleming's fluid, imbedded in paraffin, cut in serial sections of 2 to 4μ thickness and stained with Giemsa's solution or Heidenhain's iron hematoxylin, the latter being counterstained with eosin or orange G.

MORPHOLOGY OF THE TROPHIC STAGE

I expected that in sections would be found many young developmental phases of the organism which could not be observed externally in the fresh state, but in the study of the numerous sections, to my disappointment, only very few of the parasites were observed, showing that the infection in the present case was one of slight degree. The focus of infection was the connective tissue of the gill filament. The connective tissue became swollen by the infection of the parasite, and with its growth the tissue around it formed a thick layer, penetrated by numbers of capillaries (Figs. 1 to 3). A similar phenomenon has

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already been observed by Cohn in *Myxobolus minutus* and by Schröder in *Henneguya acerinae.* The parasite in the branchiae is generally ovoidal in form (Fig. 1), but sometimes a calabash-shaped one is present (Fig. 2). This is probably caused by the union of two closely neighboring individuals. The gill-filament infected is not so greatly swollen as is the case with *Henneguya acerinae, Myxosoma dujardini* according to Thélohan (1895), and *Henneguya gigantea* according to Nemeczek.

The youngest form found has an oval shape. The dimensions are about 67 by 50μ, showing clearly the differentiation of the protoplasm into ectoplasm and endoplasm. The ectoplasm exhibits vertical striations (Fig. 3), similar to those of *Myxidium lieberkühni, Myxobolus pfeifferi* according to Thélohan (1895), of *Henneguya acerinae* and also of *Sphaeromyxa sabrazesi* according to Schröder (1907), and *Myxobolus gigas* according to Auerbach. Besides this structure, in some specimens the ectoplasm differentiates very fine plasmic processes, 2 to 3μ long, from its surface (Fig. 3). Auerbach (1909) noted a structure analogous to the above-mentioned one in *Myxobolus fuhrmanni,* but he could not determine whether it belonged to the parasite or to the tissue of the host. Schröder (1907) observed a similar differentiation of the ectoplasm in *Sphaeromyxa sabrazesi,* stating that "an der Oberfläche des Ektoplasms erkannte ich bei einigen Exemplaren einen zottenähnlichen, wenig über 1μ hohen Besatz."

The endoplasm has a coarsely granulated structure. The nuclei are round or oblong, in size varying from 1 to 4μ. They are scattered in the endoplasm, unlike the nuclei observed previously by Thélohan, Schröder, etc., who found them situated rather in the middle portion of the endoplasm.

In some young specimens, where the spore formation had begun to take place, I noticed that the nuclei and the pansporoblasts took a peripheral position, while in the middle portion a large round granulated body of distinct contour, but with no nucleus in it, was observed. I could not determine whether it is an accumulation of the endoplasm or an inclusion.

In the older cyst, which is oval-shaped and of about 190μ in maximum diameter, the ectoplasm becomes thinner than in the younger form. In the periphery of the endoplasm, numbers of nuclei are to be found, and towards the middle portion of it matured spores and several developmental phases of pansporoblasts to spores.

SPORE FORMATION

The nuclei in the plasmodium may be distinguished as vegetative and generative. The latter are always found in a round cell which takes stains more deeply than the surrounding endoplasm. The uninu-

cleate cells are the "sphères primitives" of Thélohan (1895), "pansporoblasts" of Gurley, or "Propagationszellen" of Keysselitz (1908). The propagative cell is of oblong or spindle shape, though usually round in form, with dimensions of 4 to 8μ. The nucleus is often situated excentrically (Fig. 4). A caryosom, as Keysselitz mentioned, is always found in it. The propagative cell multiplies by division into two or three daughter cells (Figs. 5 to 16). These points correspond to some extent with those of *Myxobolus pfeifferi* according to Keysselitz (1908) and to Mercier, and *Myxidium bergense* according to Auerbach (1912). The nuclear division in the propagative cell of *Sphaeromyxa sabrazesi* according to Schröder (1907 and 1910), *Myxobolus pfeifferi* according to Keysselitz and to Mercier, and *Henneguya psorospermica* according to Auerbach, is reported to be mitotic. In the present form I also observed mitotic division. The chromatin, through the coil stage (Figs. 5 to 7), divides into two parts, exhibiting very often the central spindle (Figs. 8 to 10). In this respect it resembles that of *Myxidium bergense* studied by Auerbach (1912).

The propagative cells resulting from the multiplication go on to spore formation. The greater propagative cell (macrogamete) and the smaller one (microgamete) take an elongated form and associate with their lateral surfaces. At first a space is seen between them (Figs. 17 and 18), and finally the cytoplasm of both cells fuses at the place of contact (Figs. 19 to 23).

The association of two binucleate cells, observed by Schröder in *Sphaeromyxa sabrazesi* and by Keysselitz (1908) in *Myxobolus peifferi,* does not exist in the present parasite. The association of the two uninculeate propagative cells in the present Myxosporidia strikingly resembles those observed by Mercier (1904) in *Myxobolus pfeifferi* and by Auerbach (1912) in *Myxidium bergense.* But the nuclei of the associated form do not fuse into one, as Mercier thought happened in *Myxobolus pfeifferi.*

The nuclear change in the pansporoblast coincides to some extent with that mentioned by Auerbach (1912) in *Myxidium bergense.* Instead of uniting into one, the nuclei in the associated form undergo division. The smaller nucleus divides into two at the peripheral position of the pansporoblast, being destined for the nuclei of the pansporoblast (Figs. 23 to 26). The greater nucleus repeatedly divides mitotically with the growth of the pansporoblast (Figs. 22, 25 to 31). In the fully developed pansporoblast, ten nuclei are observed, besides two nuclei of the pansporoblast and the reducing nuclei. At this stage, the contents of the pansporoblast separate into two sporoblasts, each of which contains five nuclei (Fig. 31). Of the five, two are found in a plasmic mass (sporoplasm in the later stage), one is in a cell which usually has a vacuole in it (nucleus for polar capsule and polar fila-

ment), and the remaining two are for the spore membrane. They are clearly recognizable in young spores, as is shown in Figures 32 to 37. When the spore is fully developed, the membrane of the pansporoblast is broken up, and the spores consequently become free in the endoplasm as in bicapsulated Myxobolus according to Keysselitz. As I mentioned above, we always recognize several developmental stages of the spore in the older cyst.

MORPHOLOGY OF THE SPORE

The spore has a pyriform shape, with a peculiar attenuated anterior and broadly rounded posterior extremity (Figs. 38 to 45). It has no bilateral symmetry. The spore-membranes of *lateral surfaces* are usually curved in opposite directions (Figs. 39 and 40). The form agrees well with that of *Myxobolus piriformis* described and illustrated by Balbiani and by Thélohan (1895). Spores of the calabash shape, however, occur not infrequently in the present case (Fig. 38). The spore-wall is comparatively thin and composed of two valves, superior and inferior. At the plane of junction the shell is somewhat thickened (Figs. 41 and 44). The surface of the spore usually represents no special structure. Very rarely a single, short, tail-like process about 1.5μ in length is seen at the middle part of the posterior end (Fig. 41). Thélohan (1895) observed a similar abnormality of the spore in *Myxosoma dujardini* and described that "quelques spores anormales ont un prolongement caudal." I also regard the above-mentioned process in certain spores as an abnormality. The length of the spore is about 15μ, the breadth 7 to 8μ and the thickness 5 to 6μ. Thélohan gives the size of the spore of *Myxobolus piriformis* to be 16 to 18 by 7 to 8μ. In the fresh preparations one pyriform polar capsule is observed at the anterior half portion of the spore (Figs. 42 to 44), its dimensions beings 7 to 8 by 3 to 4μ. The wall is drawn out anteriorly into a minute duct which pierces the shell near its anterior extremity, affording exit for the polar filament. Thélohan did not measure the size of the polar capsule of *Myxobolus piriformis*. But it seems to be much smaller than the present form (compare his Figures 116 and 117, Plate IX, 1895, with my Figures 38 to 45). Auerbach (1909) observes spores with two polar capsules among unicapsulated spores of *Myxobolus fuhrmanni*. In the present case, all spores have only one large polar capsule each, of which I will speak again when I come to the permanent preparations. Moreover, in some spores, the nucleus of the polar capsule is seen to be attached to it (Figs 42 and 43). The polar filament is easily extruded from the anterior end of the polar capsule when the spore is treated (Fig. 45) with a reagent like caustic potash, or hydroxyl, or pressed mechanically between the cover and slide glasses. The length of the filament measured after the spore has been

freshly prepared and pressed agrees usually with the measurements of stained ones prepared according to my method (1913). The length of the polar filament of the parasite is 40 to 45μ, so it is 10 to 15μ longer than that of *Myxobolus piriformis* measured by Thélohan. The posterior half portion of the spore is filled with sporoplasm. In fresh preparations, it is of a transparent, somewhat granular structure. Treated with iodin-alcohol, there appears a large vacuole stained brownish yellow in the sporoplasm.

In fixed preparations, the anterior end of the spore-membrane is stained very faintly (Figs. 38 to 41). The duct of the polar capsule becomes easily visible. In some spores, close to the anterior end of the polar capsule, there is an oblong mass of protoplasm (Figs. 38 to 40). I took this structure at first to be a polar capsule and compared it with the "Körperchen" of *Pimelodus blochii* of Müller, i. e., *Myxobolus inequalis* described by Gurley. But no such structure is observed in my present preparations, so that I cannot determine whether it is a degenerating polar capsule or some other structure. The nucleus of the polar capsule is always observed in young spores, well stained at the peripheral part of the capsulogenous cell (Figs. 33 to 37). In the sporoplasm, a large iodophile vacuole remains unstained, its diameter being about 3μ. The iodophile vacuole of *Myxobolus piriformis* observed by Thélohan is smaller than the present one. Two nuclei are always found in the sporoplasm situated closely to each other. They are usually of equal size (Figs. 37 to 41), but sometimes of different dimensions (Fig. 37). The position of the nuclei in the sporoplasm is not always the same. They are seen between the polar capsule and the vacuole (Fig. 39), on a *lateral aspect* of the vacuole (Fig. 38), or near to the posterior end of the spore (Figs. 40 and 41).

To what genus and what species does the present parasite belong? Because of the presence of an iodophile vacuole, it is clear that it belongs to the genus Myxobolus. So far as I am aware, the unicapsulated Myxosporidia known up to the present time are four in number, all belonging to the genus Myxobolus:

Myxobolus piriformis Thélohan
Myxobolus unicapsulatus Gurley
Myxobolus fuhrmanni Auerbach
Myxobolus oculi-leucisci Trojan

Of these, *Myxobolus unicapsulatus* is quite different from the present form. If one compares Figures 5, Plate 16 of Müller (1841) with my Figures 38 to 41, one sees great differences between the spores. Moreover, the habitat is quite different. *Myxobolus fuhrmanni* as stated by Auerbach (1909) was found in the connective tissue of the mouth of *Leuciscus rutilus* L. The spore is much larger than the present one

Explanation of Plates

All figures except Nos. 1 and 2 are drawn with Abbe's drawing camera.

Figs. 1 to 41 from sections.

Figs. 42 to 45 from fresh preparations.

Staining: Figs. 7, 9, 14, 17, 18, 21, 22 and 35: Giemsa's solution.

All the others: Heidenhain's iron hematoxylin and eosin.

PLATES I AND II

Figs. 1 and 2.—Parts of longitudinal sections of infected branchial lamellae, showing the seat of the parasite. 1, × 160; 2, × 320.

Fig. 3.—A peripheral portion of the parasite, showing the differentiation of the protoplasm. × 1000.

Fig. 4.—A propagative cell from the plasmodium. × 2250.

Figs. 5 to 16.—Division of the propagative cell. × 2250.

Figs. 17 to 21.—Association of the macro- and microgametes. × 2250.

Fig. 22.—Nuclear division of a macrogamete. × 2250.

Figs. 23 and 24.—The same of the microgamete. × 2250.

Figs. 25 to 30.—Several developmental stages of the pansporoblast. × 2250.

Figs. 31 and 32.—Segmentation of the pansporoblast into two sporoblasts. × 2250.

Figs. 33 to 37.—Young spores in development. × 2250.

Figs. 38 to 41. Matured spores. × 2250.

Figs. 42 to 45—Spores from fresh preparations. × 1000.

Fig. 45.—A spore with polar filament extruded. × 1000.

PLATE I

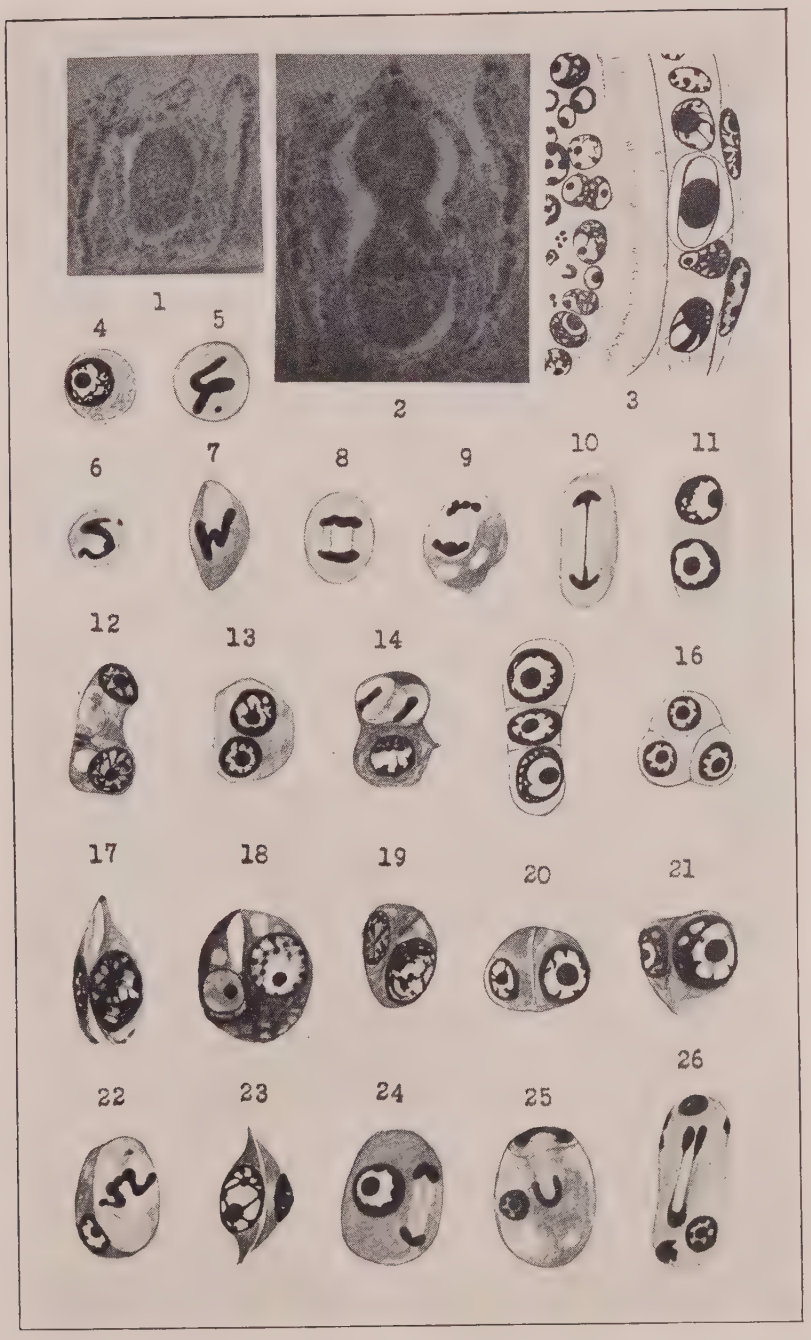
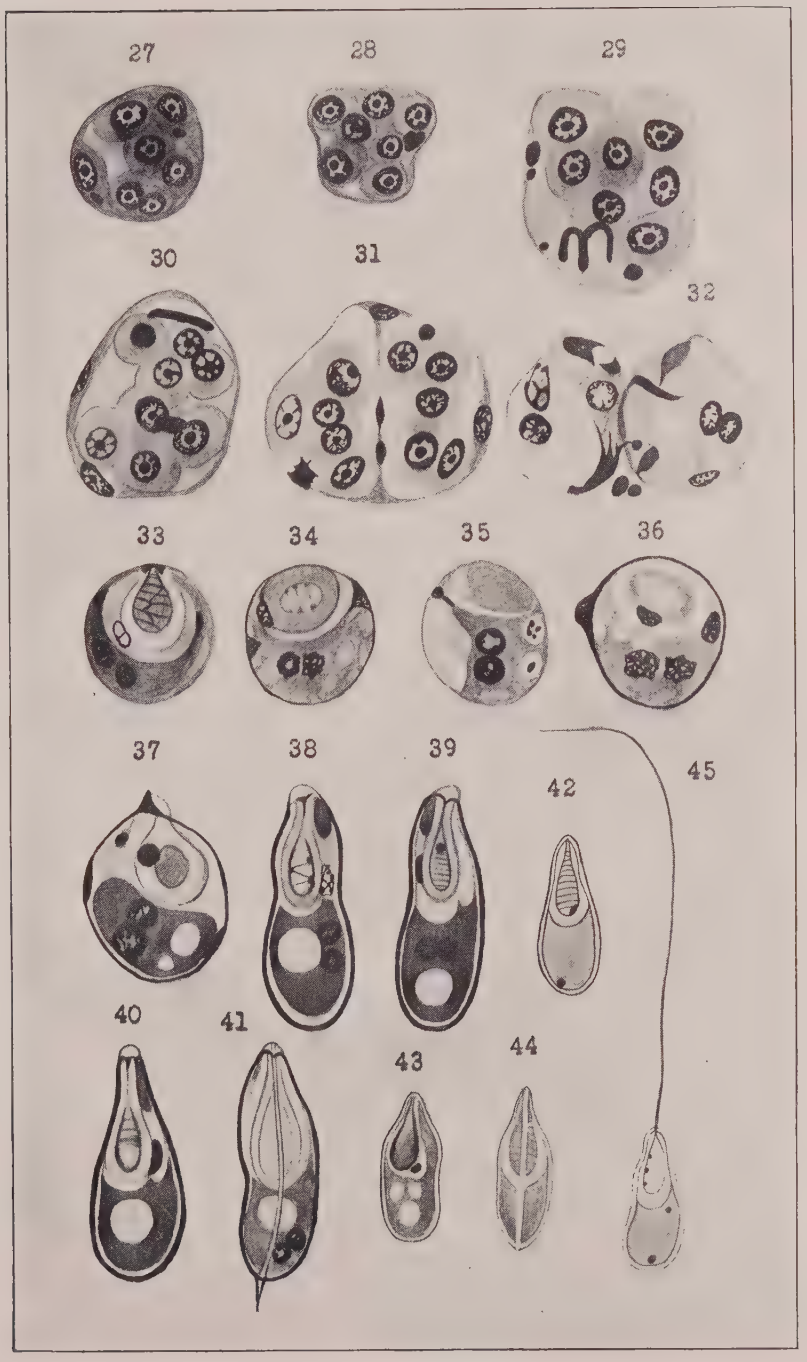


PLATE II



(length 18 to 20μ; breadth, about 8μ; thickness, 6μ, and the length of the polar capsule, 9 to 10μ). The spore membrane is thickened at the posterior end and has 4 to 6 notches. None of these points agree with the observations mentioned above on the present Myxobolus. The same is true of *Myxobolus oculi-leucisci,* which was found according to Trojan (1909) in the vitreous humor of the eye of *Leuciscus rutilus* L. Though the size of the cyst is almost equal to my parasite, the spore is smaller and different in structure.

I have spoken only partially of the comparison between the present Myxobolus and *Myxobolus piriformis,* and will compare them here again in the following synopsis:

	Myxobolus piriformis	The present Myxobolus
Habitat	Branchiae and spleen of *Tinca tinca* L.; kidney of *Misgurnus fossilis*	Branchiae of *Cyprinus carpio* L.
Cyst	"Les kystes branchiaux de cette espèce se reconnaissent à leur minceur: il forment de petites stries filiformes et non des tumeurs sphériques comme le *M. ellipsoides*" (Thélohan, 1895: 348)	Small round cyst in the connective tissue of the gill-filament
Spore:		
Form	Pyriform, with attenuated anterior extremity	Pyriform, with attenuated anterior end; often calabash form
Size	Length 16 to 18μ Breadth (Max.) 7 to 8μ	Length 15μ Breadth 7 to 8μ Thickness 5 to 6μ
Polar	Undescribed, figured only, capsule seems smaller	7 to 8μ by 3 to 4μ
Iodine vacuole	Smaller	About 3 to 4μ in diameter

As will be seen from the above comparison, there are great differences in the form of the cyst, the host, the size of the polar capsule, and the length of the polar filament, though the form and dimensions of the spore resemble each other.

Hence I think the Myxobolus found by me is a new species, and propose to call it *Myxobolus toyamai* nov. spec. in honor of Prof. Dr. K. Toyama, who kindly introduced me to this branch of protozoology in the year 1909.

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Note. This paper was printed in Japanese in 1915 and is reprinted here at the request of the author.

THE OCCURRENCE OF *BOTHRIOCEPHALUS LIGULOIDES* LEUCKART, WITH ESPECIAL REFERENCE TO ITS DEVELOPMENT

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This larval cestode was first discovered by P. Manson at the post-mortem examination of a Chinaman at Amoy in 1882, and was described as *Ligula mansoni* by Cobbold in the following year. Since then about fifty-five cases have been reported, mostly from Japan with the exception of a few cases from Africa and the Malay Archipelago. The cases were all reported from the human host and it has been questioned for a long time whether this cestode larva was not confined to the human host. Some authors have suspected the existence of this worm in other animals without having actually proved its occurrence outside of man. A very few writers have described unsatisfactorily and uncertainly the occurrence of the cestode larva in question in animals. For instance, Dr. H. Miyake found twelve specimens of a cestode larva in the muscles of a monkey which had recently died, and he reported his belief that they were the same species as the liguloid larva from the human host, comparing them with the specimens and descriptions of previous authors. But the lack of a precise description for his own specimens prevented their positive identification. Other authors also, viz., A. Hirohata and J. Maejima, have proved experimentally that the larva is able to live and grow in the body of the rabbit by transplanting small pieces of the worm with a scolex into the body cavity of that host.

In 1915, during my animal experiments with the encysted larvae of the lung distome, I accidentally came on August 11 across thirty-six specimens of this larval cestode in the body cavity and body wall of the cat employed in my experiment, which died in an extremely anemic and undernourished condition. Some worms were enclosed by a thin fibrous membrane, while others were lying free in the body cavity or in various tissues of the host. One, two, or even three worms were found in one capsule, and the latter were generally smaller in size than the former. The capsules lay in the muscular or subcutaneous tissues, varying in size and shape. A particularly large number of worms were found in the abdominal and pleural muscular wall, where they were tangled together into a ball or were creeping about here and there.

Some portions of the body wall occupied by the worms had suppurated. This agrees with the suppurating condition which is often

reported by various authors for human patients suffering from this cestode larva. Some worms were wound and twisted through various parts of body in such a manner that one end lay in the abdominal cavity and the other end in the abdominal wall, whereas the median portion of the worm lay irregularly in the body cavity and body wall. This state in the body of the host obviously proves the migratory tendency already frequently observed in the human host.

Fresh specimens were extremely mobile, especially in warm physiological salt solution, varying actively the shape and size of body. Large specimens measured 40 to 75 cm. in length and 17 to 20 mm. in breadth. There were also many other specimens in various stages of development or growth. Even in the same individual the length and breadth varies considerably according to the state of contraction. Generally, when it was killed by a fixing agent such as a hot saturate solution of corrosive sublimate, the worm contracted to two-thirds the length of living specimens.

From my own observation of the morphological character and anatomical structure of this worm, as well as its identification by Prof. Dr. Ijima, who was the first writer to describe this cestode larva in Japan, it is evident that the worm in question is the same as Manson's larval cestode of man. Thus I have proved the actual occurrence of this cestode larva in an animal. Furthermore, I am inclined to believe that the normal intermediate host of this tapeworm should not be sought in a human being, but in another animal, though the parasite has not been found previously in the latter host while it has been found so often in the former. Why it seems to occur so often in the human body and so seldom in other animals doubtless depends upon the fact that human parasites are sought more carefully and are hence more frequently found than those of animals. It is obvious that the further development of this cestode larva would be impossible, or less likely at least, if the larvae were normally confined to the human body as a natural intermediate host. I am of the opinion that many animals, domesticated and wild, will be discovered to act as the intermediate host of this larval cestode.

About six months after my discovery, M. Sugimoto in Formosa reported cestode larvae from the pig as *Both. liguloides,* and added that his specimens were quite similar to and probably identical with the specimens of *Sparganum raillieti* Rátz 1913 from the pig. The descriptions of Rátz's and Sugimoto's specimens indicate their likeness. But it is doubtful whether they are identical with Manson's larval tapeworm from man or my specimen from the cat.

As stated above, the great majority of the cases infected with this cestode larva have been reported from Japan. They were found in various districts throughout the country, but especially often near

Osaka, the section where thirty-three out of fifty-five cases (60 per cent) are recorded. Thus I am now in a favorable locality to study the worm in question. Many difficulties, however, are encountered in studying the worm, because it occurs very rarely and consequently is found only accidentally in the human body or in other animals. In spite of efforts by various authors at various times, nothing is known of the life-history of the parasite.

I tried twice animal experiments to determine the final host of this larval cestode. In the first case I used two young cats as hosts and the cestode larva from the cat mentioned above. On August 11, 1915, two larvae were fed to one young cat and one larva to the other. The cats unfortunately died on the 17th of the same month from some unknown cause, and on dissection, no parasites of any kind were found in the alimentary tract.

In the second case I made the experiment with the cooperation of my colleague S. Yamada. On June 26, 1916, a specimen of this cestode larva was obtained from a patient who suffered from the worm in the left side of her abdominal wall. The specimen measured 15 cm. in length and 5 mm. in breadth, and was enclosed in a capsule. This larva was given through a catheter to a young dog which was 27 days old, and had been reared in our anatomical department. Before the feeding, the feces of the dog were repeatedly examined for parasite eggs, and we found the eggs of *Dipylidium caninum* only. Afterwards we examined daily for parasite eggs; and on the thirteenth day after experimental feeding we first found a new kind of parasite egg which increased in numbers day after day, and ultimately attained a maximum condition that continued until the animal was killed.

The eggs are elongated oval in shape, tapering toward both poles, markedly sharper at the anterior than at the posterior end. On the anterior pole they are provided with a small operculum. They are mostly symmetrical, the curvature on the two sides of the long axis being unequal. One may find a minute globular thickening of the egg-shell at the posterior pole in some eggs, such as is observed in the eggs of certain distomes. The eggs closely resemble those of Dibothriocephalus, but are darker brown in color and different in shape. Some measurements in microns are as follows:

	1	2	3	4	5	6	7	8	9
Length	76.9	75.8	74.5	69.3	68.7	68.5	64.5	64.5	62
Breadth	43.5	37	33	37.1	38.7	36.3	41.9	37	37
Ratio	1.76:1	2.04:1	2.25:1	1.86:1	1.77:1	1.88:1	1.53:1	1.74:1	1.67:1

On August 26, over two months after the feeding, we killed the dog and examined it for the parasites. We found a few specimens of Ancylostomum, two of *Dipylidium caninum*, and one large tapeworm

belonging to the genus Dibothriocephalus. This measured 2.5 m. in length and 12 mm. in maximum breadth, the maximum length of proglottis being 2 mm. When alive it was very active and its length and breadth varied considerably, as is usual among cestodes. The posterior extremity showed a bifurcated anomaly, the body being divided into two halves near the median line, one half being 80 mm. long by 5 mm. broad, and the other half 50 mm. long by 3 mm. broad. The length of a proglottis in the bifurcated portion was constant (2 mm.).

From observations on the external features and the internal structure we easily identified this specimen as belonging to the genus Dibothriocephalus, and bearing a close resemblance to *Dib. latus*. But I doubt whether the worm is identical with *Dib. latus* of the dog previously reported. The known species of the genus Dibothriocephalus from the dog are *Dib. fuscus* Krabbe 1886, *Dib. serratus* (Diesing,

Eggs of dibothriocephaloid cestodes from the various hosts. × 620.
A. From human host. B. From our dog used in experiment. C. From lion.

1850), *Dib. cordatus* Leuck. 1863, and *Dib. latus* (L. 1748). The worm in question may easily be distinguished from any of the first three species mentioned above.

I will add a few words on the comparison of this worm with *Dib. latus* which it resembles in some characteristics and not in others. Resemblance exists in respect to scolex form (though not accurately observed on account of irregular distortion by contraction), general form of the strobila, proportion of length and breadth of the proglottis in every part of the strobila, and general structure of internal organs and tissues. A remarkable point of difference is in the shape of the eggs. The eggs of this worm are easily distinguished from those of *Dib. latus* by their shape and the ratio of length to breadth.

The eggs of the new worm are elongated oval in shape and mostly asymmetrical, the curvature on both sides of long axis being unequal, and they taper toward both poles, ending slightly pointed. The anterior

pole is more pointed than the posterior, as stated above. The proportion of length to breadth, varying from 1.53:1 to 2.25:1, is greater than that (1.16:1 to 1.48:1) of *Dib. latus*. The eggs of *Dib. latus* are oval, both poles ending equally rounded and relatively broader than those of the new worm.

Measurements show that there is a great variation of egg size in *Dib. latus* according to the species of the host, whether human or other animal. The eggs of *Dib. latus* from other animals are the same in shape but much smaller than those from the human host. The next table serves to show the variation of egg size.

	Human 1	Human 2	Human 3	Lion 1	Lion 2	Lion 3
Length	74.2	69.3	66.1	58.1	54.8	54.8
Breadth	51.6	46.7	50	35.4	35.4	33.8
Ratio	1.16:1	1.48:1	1.32:1	1.62:1	1.54:1	1.62:1

From the above tables it is evident that the eggs of the new worm are midway in size between those of the tapeworms from the human host and from the lion.

In spite of such a great difference in the size of eggs, dibothriocephaloid cestodes from human host and other animals have been considered to be the same species as *Dib. latus* by all previous authors. If this identification by the previous authors is unquestionable, the new worm might be identified as *Dib. latus* and the remarkable difference in size and shape of eggs be considered a mere variation among the same species. If this supposition is right, one must reconsider the animal experiment. If the worm obtained from the dog under experimentation is supposed to be *Dib. latus,* the dog must have swallowed a larva of this cestode species; that is, have eaten the raw meat of salmon trout, which is considered in Japan to be the only species of fish harboring the larval form of this cestode. During our experiment the dog was always kept in a cage and fed regularly, so that he never obtained fish as food or accidentally. Before the experiment the dog was nursed by the mother or fed upon remnants of food which were generally boiled or roasted and could not be expected to contain a living larval cestode. Such a careful feeding experiment makes it impossible to think of an accidental infection with *Dib. latus* by food containing the larva. Therefore I am doubtful that the worm is surely identical with *Dib. latus,* and consequently it is a question whether the dibothriocephaloid cestodes from the human host and from other animals have been correctly identified by previous authors.

There is only one remarkable character, the egg size and shape, useful to distinguish positively the worm from the known species, *Dib. latus*. Egg size and shape of parasites, however, is generally assumed

to play an important rôle in determining species. Agreeing with this point of view supported by H. B. Ward, A. Looss, and other helminthologists, I have the following opinion in respect to the eggs: I am inclined to believe the worm in question will be experimentally determined hereafter to be the matured form of Manson's larval cestode and quite different from *Dib. latus*. Consequently, some cases — especially from natural infection — of supposed *Dib. latus* from other animals such as the lion, dog, cat, etc., previously reported might have been mistakenly identified and really might have been the mature form of Manson's larval cestode, or indeed of still another species.

The natural mode of infection by *Dib. latus* also seems to support my supposition. To become infected with *Dib. latus,* it is necessary to eat raw fresh meat of fish harboring a larval form of this tapeworm; such fish are the salmon trout in Japan, or pike, salmon, perch, etc., in Europe. Generally the dog is fond of uncooked meat, but not of fish, in Japan at least; so it is unnatural and very rare for dogs to get fresh fish as food. Therefore it is puzzling to me why dogs are infected so often with this tapeworm in Japan, especially in the districts where the fish intermediate host is not found. The same difficulty holds good for the case of the lion, tiger, and other animals which are frequently infected with *Dib. latus,* although these wild beasts are accustomed to eat other weaker beasts and birds, but not fish so far as we know.

It has already been proved, however, by previous authors that the plerocercoid larva of *Dib. latus* from fish can develop to the adult form in the alimentary tract of dog and cat. So it is probable, I think, that the dog and perhaps other beasts like the lion, tiger, etc., can become infected with two kinds of dibothriocephaloid tapeworms, viz., the well-known species *Dib. latus,* and a new species, the adult form of Manson's liguloid larva.

At any rate to have found the adult form of Manson's larval tapeworm is both important and interesting, not only as a contribution to the knowledge of the development of the worm itself, but for the determination of species of dibothriocephaloid cestodes from the human host and from animals.

In closing, I wish to express my appreciation to Prof. Dr. Ijima, Chief of the Zoological Institute, Tokyo Imperial University, for his kind identification of the cestode larvae, and to Prof. Dr. Sakurane, Chief of the Dermatological Department of our hospital, for his courtesy in placing the material at my disposal.

A FURTHER NOTE ON THE LIFE-HISTORY OF *GONGYLONEMA SCUTATUM* *

BRAYTON H. RANSOM AND MAURICE C. HALL

In two recent papers, one of which is an admirable monograph of the larval forms of the heteroxenous parasitic nematodes and the other a comprehensive study of the Gongyloneminae of North Africa, Seurat (1916: 739; 1916a: 358) has expressed the opinion that certain larval nematodes found in various species of coprophagous beetles (Aphodius, Onthophagus) which we identified (Ransom and Hall, 1915: 154; 1916: 80-86) as the larvae of *Gongylonema scutatum* probably belong to another species, *G. mucronatum* Seurat 1916. The adults of the latter species have been found by Seurat in the mucosa of the esophagus and base of the tongue of the Algerian hedgehog (*Erinaceus algirus* Duv.). It has not yet been recorded from sheep or cattle. Easily recognizable differences between *G. scutatum* and *G. mucronatum* are as follows, the statements relative to structural characters of the latter being taken from Seurat's description:

In *G. scutatum* the cervical papillae are situated about midway between the anterior border of the nerve ring and the anterior end of the body, each in the center of a rounded cuticular shield; in *G. mucronatum* they are situated at the anterior third of the distance between the anterior border of the nerve ring and the anterior end of the body and are not inserted in the center of a cuticular shield. In *G. scutatum* the caudal pores are subterminal, in *G. mucronatum* situated at about two-thirds of the distance between the anus and the tip of the tail. In *G. scutatum* there is no papilla in the neighborhood of the vulva; in *G. mucronatum* an unpaired papilla is situated on the ventral surface of the body about 0.1 mm. behind the vulva.

In view of Seurat's opinion we have examined in detail numerous specimens of Gongylonema collected from the esophagus of sheep and cattle in various parts of the United States and have failed to find among them any corresponding to *G. mucronatum,* or to any species other than *G. scutatum.* If *G. mucronatum* is present in the United States, it is not likely that it occurs in sheep or cattle; at least, it must be rare in these hosts. Consequently, even considering the fact that we did not make a detailed microscopic examination of every worm from which eggs were obtained for feeding to insects in the experiments recorded in our former paper, but depended in many instances

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upon the gross appearance of the parasites as sufficient for their identification, it seems scarcely possible that there should have been invariably present in the material fed to the insects not only the eggs of *G. scutatum,* but also those of another species, whose presence in the sheep or cattle from which our material was obtained we constantly overlooked. The only apparent possibilities of error affecting our interpretations of the results of our experiments in feeding croton bugs and beetles in addition to the one just mentioned are (1) that the insects were already infested, and (2) that during the progress of the experiments they acquired the parasites from some other source than the material originally fed to them. In the case of the beetles we realized that some of them probably already harbored the parasites at the beginning of the experiments and gave due consideration to this probability in interpreting our observations; but the possibility of such a circumstance in the case of the croton bugs is very slight in view of the fact that we have frequently examined croton bugs caught from the same places as those used in the experiments without finding Gongylonema larvae. The second possibility is also very slight in the case of the croton bugs, as they were kept during the experiments either without access to food or fed only on bread crumbs or similar food unlikely to contain nematode eggs. Furthermore, croton bugs kept in a similar manner and used in other experiments have never shown Gongylonema larvae. The beetles used in our experiments in some instances were kept in containers with unsterilized feces from sheep and consequently might have acquired their parasites from this source, but in certain instances the feces in which the beetles were kept and upon which they fed were sterilized. In the latter case, even with beetles already infested, one would be justified in considering as we did that the newly hatched Gongylonema embryos observed in large numbers a day or two after feeding, and the developing larvae in progressive stages observed later in due course of time, came from the eggs contained in the material fed to the beetles. So far as we are able to perceive after reviewing our records and recollections, our experiments in the feeding of Gongylonema eggs to insects were adequately safeguarded and controlled in all essential respects, with the exception that possibly sufficient care was not taken to exclude the eggs of species other than *G. scutatum.* With this possibility of error in view, slight though it is, the senior author has carried out a new series of experiments in feeding croton bugs.

The insects used in the recent experiments were kept in flasks closed by cotton plugs. In addition to being supplied with material containing the Gongylonema eggs they were occasionally given fresh bread crumbs and a few drops of water. Numerous individuals caught from time to time in the same place as those used in the experiments

were examined and found to be free from infestation. Some were also kept in flasks and fed bread and water, but no Gongylonema eggs, as controls against those fed Gongylonema eggs. The controls remained free from infestation. The Gongylonema material for feeding was obtained from a few infested gullets of sheep and cattle procured at an abattoir. All of the parasites were carefully removed from the gullets. By microscopic examination the fact was established that all of the worms present in each of the gullets corresponded to *G. scutatum,* special attention being given to the cervical papillae, absence of a postvulvar papilla, and subterminal location of the caudal pores. Female worms thus obtained and identified were washed in several changes of physiological salt solution to reduce the chances of foreign eggs adhering to their bodies, cut into small pieces, mixed with bread crumbs, and placed in the flasks containing the captive croton bugs. As in our former experiments, these croton bugs became infested with the larvae of Gongylonema. Individuals were examined at intervals, and various stages ranging from the newly hatched larvae up to the encysted forms, and exhibiting the same characteristics of structure as those described in our former paper, were recovered. An encysted larva taken from a croton bug seven weeks after eggs of *Gongylonema scutatum* were placed in the flask in which it was kept, measured 1.9 mm. in length by 0.06 mm. in diameter. The pharynx was 0.035 mm. in length, the esophagus 1.2 mm., its muscular portion 0.23 mm. The cervical papillae were 0.07 mm. from the anterior end of the body, the nerve ring 0.125 mm., excretory pore 0.21 mm. The anus was 0.09 mm. from the tip of the tail, the caudal pores 0.025 mm.

With reference to our experiments in feeding sheep with infested beetles and croton bugs (Ransom and Hall, 1916) it may be noted that their results if considered by themselves were less conclusive than those of the experiments in feeding Gongylonema eggs to insects, because of the small number of animals used and the lack of complete control of all the conditions which might have affected the experiments. Furthermore, no attempt was made to obtain a series of steps in the development in sheep between the larva and the adult. As a matter of fact in our former paper we did not insist upon the conclusiveness of the sheep-feeding experiments and considered them of importance only when viewed in the light of other evidence without which they would have been much less significant. Even though the experimental evidence that the larvae of *Gongylonema scutatum* in dung beetles develop to maturity in sheep and other suitable mammalian hosts when the insects are ingested by these animals is less complete than that as to the development of the larval stage in the insects, such evidence as we have is in exact accord with that hypothesis, which moreover by analogy is strongly supported by the known facts in the life histories

of other parasites, and we are justified in assuming until very definite evidence to the contrary is brought forward, that sheep, cattle, and other suitable host animals become infested with *Gongylonema scutatum* as a result of swallowing infested insects, under natural conditions probably various species of dung beetles.

From the foregoing it is evident that the validity of the results of our work on the life history of *Gongylonema scutatum* has not been affected by the question raised by Seurat regarding the correctness of our identification of the larval nematodes which we found in coprophagous beetles and under experimental conditions in croton bugs. It is also evident that the nematodes found by Seurat (1916: 739, Fig. 5; 1916a: 315, 346) in several species of Blaps, and because of the subterminal position of the caudal pores considered by him to be the larvae of *G. scutatum,* cannot belong to this species, unless it is a species whose larvae are characterized by an unusual degree of polymorphism. Whether the nematodes occurring in various species of coprophagous beetles in Algeria, which are strikingly similar to those which we have shown to be the larvae of *G. scutatum,* belong to *G. mucronatum* as Seurat (1916a: 317, 346, Fig. 11) supposes, remains to be determined. The basis upon which Seurat identified them as *G. mucronatum* is the location of the caudal pores at a considerable distance from the tip of the tail, a character in which they agree with the adults of this species. Clearly, however, apparent similarities in details of structure are not sufficient in the absence of other evidence to justify definite conclusions as to the specific identity of larval and adult nematodes, and further investigations will be necessary before the larvae described by Seurat as such can be accepted as the larvae of *G. mucronatum.* Because of their close agreement in structure with the larvae of *G. scutatum,* it is quite probable that they actually belong to this species, the adult stage of which Seurat has found to be common in Algeria.

SUMMARY

Despite the doubts raised by Seurat in recent publications, the conclusions expressed in our former papers on the life history of *Gongylonema scutatum* are still valid. It has been definitely proved that dung beetles and croton bugs fed upon the eggs of *G. scutatum* become infested with an encysted larval stage of the parasite, and the evidence is very strong, if not quite conclusive, that sheep, cattle, and other suitable mammalian hosts become infested as a result of swallowing infested insects (usually under natural conditions, various species of dung beetles).

The nematodes found in several species of Blaps in Algeria and identified by Seurat as the larvae of *G. scutatum* belong to some other species.

It is not improbable that the nematodes found in Algerian beetles which Seurat has considered to be the larvae of *G. mucronatum* in reality belong to *G. scutatum*.

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1916a. Contribution a l'étude des formes larvaires des nématodes parasites hetéroxènes. Bull. scient. de la France et de la Belg., (7) 49: 297-377; figs. 1-14.

NOTES

The intermediate host of *Schistosoma mansoni* in Venezuela is discussed in a recent important paper by Drs. Juan Iturbe and Eudoro González. By infection experiments with the mollusks of the valley around Caracas, the true intermediate host was found to be *Planorbis guadelupensis*. The miracidia of *S. mansoni* developed into sporocysts within this host, and subsequently furcocercous cercariae were obtained from it. By immersion in water infected with these cercariae and by feeding experiments white mice were brought to develop adult *S. mansoni*. The paper is illustrated with two microphotographic plates.

A recent number of Japanese Medical Literature states that *Schistosoma japonicum* is reported by Narabayashi to depend on a small snail in the rice fields as its intermediate host. According to Pilsbury this snail should properly be called *Blanfordia nosophora* Robson. The cercariae invade the skin even if the latter is only damp. A definite relation exists between schistosomiasis and a skin disease called "kabure."

The same journal reviews a paper on *Paragonimus westermanii* in the Korean Medical Society Journal in which Muneta records from an autopsy the abundant occurrence of the fluke cysts in the abdomen under the peritoneum; liver, spleen, heart, sternum and the cheek were also invaded. Some nodules contained adult worms; others did not.

Davainea formosana, a new human tapeworm from Formosa and Tokyo, is described by Akashi in the Journal of the Formosa Medical Society and abstracted in Japanese Medical Literature. The specimens came from children. The species may be distinguished from the other member of the same genus long known as a human parasite by the following characters:

	Davainea formosana	D. madagascarensis
Length of body	43 cm.	25 to 35 cm.
Number of joints	Over 700	500 to 700
Hooks on suckers	None	Armed
Adult segment	2.0 to 2.5 by 1.0 mm.	2.0 by 1.4 mm.
Egg masses	300-400	120-150
Size of egg masses	0.26 by 0.13 mm.	0.3 mm.
Size of eggs	99 by 46μ	40μ
Size of onchosphere	12 to 14μ	15μ

"ECHINORHYNCHUS MONILIFORMIS" IN NORTH AMERICA

My attention has been called to the fact that in the Proceedings of the Philadelphia Academy (1874:76) is recorded with brief comments an exhibit by H. C. Chapman of specimens of *Echinorhynchus moniliformis* from the alimentary canal of the fox squirrel (*Sciurus vulpinus*). Stiles and Hassall also in their Preliminary Catalog of the Parasites, etc., (1894:352) list the species from *Sciurus niger* as found in the Leidy Collection. The statements in my note must be corrected in accordance with these facts. No data are given in these brief records to determine whether the authors mentioned above had before them the true European species or the North American form which I have studied.

H. B. W.

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